PB-215 137

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Public Health Service
Cincinnati, Ohio

1939

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# THE MICROSCOPIC FLORA AND FAUNA OF TREE HOLES*

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There are few if any natural environments on this earth without their inhabitants. This applies either to mammals or protistan organisms; the snow-fields and the hot springs have algal populations, and the most acid streams may support a rich microscopic life.

In 1936, the writer was investigating the breeding of mosquitoes in North Alabama. In an old limestone sink whose shallow central pond was occupied by a grove of Tupelo gum (Nyssa aquatica) some twenty tree or stump holes were found in an area of about an acre. Most of these contained water, and various ones of them were breeding places for six species of mosquitoes. Because one of these mosquitoes, Aedes thibaulti Dyar and Knab, is relatively rare, and practically nothing was known of its breeding habits, a careful study was made of the flora and fauna of these tree and stump holes, (1). Since then, additional tree holes in Ohio have been investigated and their interesting inhabitants studied.

Tree holes that contain water are rare. In the spring, or after a long rainy spell, many will be partly filled, but most frequently they will either be filled with dirt and debris, or else the wood is too porous to hold water. Occasionally one will be found which is in effect a permanent pool. With reference to their contained water they may be grouped roughly as those in the tops of living stumps, those in crotches or in the tops of protruding limbs or knots, and those in the sides of trees or limbs. The first two types may catch considerable rain water, but the last usually gets only small amounts

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* Prepared for presentation at meeting of Ohio Academy of Science, Cincinnati, Ohio, April 14, 1939.

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of water which runs down the side of the tree.

Holes so far studied, with few exceptions, have contained rain water, that is, have been free from contamination by surface ground water. But their fluid is rarely clear; either enough extracted material is picked up in running down the tree trunk, or else the debris and rotting wood in the hole provides enough so that a brown to black color obtains. Suspended materials are rare; the few determinations made of dissolved oxygen have shown variable, but usually a high percentage of saturation. Light fluctuates with the size of the hole opening. Chemical analyses have not been made, beyond determinations for tannates and hydrogen ion concentration. Often a rather "sour" smell has been noted, and it was anticipated from this and because of suspected tannic acid that the pH would be on the acid side. However, hydrogen ion concentrations lower than pH 5.8 were not found, and in a few cases slight alkalinities obtained. For these latter, the strong odors indicated the presence of putrefactive processes yielding amines. Temperatures tended toward uniformity, that is, warmer in winter than surface ground waters and decidedly lower in summer. No macroscopic animals except insect larvae and mites have been found in any of the holes so far examined. In a few, weeds had sprouted and some contained moss.

This report covers observations on 26 tree holes, all in living trees and distributed as follows: Tupelo gum, 13; Black gum, 1; American elm, 3; Maple, 3; White oak, 2; Blackjack oak, 1; Red oak, 1; Sycamore, 1; and Sweetgum, 1. In addition, four tree holes containing water were examined and no living protozoa were found. In Table No. 1 are tabulated the data observed. Material from one of these latter subsequently developed a thriving culture of Polytoma

uvella Ehrenberg. No observations were made on material from the remaining three. Three of the tree holes were periodically flooded by water from the pond in which the Tupelo gums were growing, and it is probable that when the Little Miami River is excessively high, the hole in the Sycamore is flooded.

Entrances to the holes varied from about eight inches in diameter to about one-half inch. One hole which contained water whenever visited, was about 20 inches deep, and two inches in internal diameter, with an opening about 3/4 inch in diameter. It usually had about 8 inches of water and an inch of debris on the bottom.

About 140 species of algae and protozoa were recovered from these 29 holes. Since most of the holes were visited but once and since cultures from them usually developed additional species under laboratory conditions, it seems probable that a much larger list of organisms could be compiled. The hole which gave the greatest number of species was Number 3, which showed 63 species. This hole was periodically flooded, but its organisms were more abundant than in the pond, and many of them were not common to the pond. On one occasion its water was deep brown, due to enormous numbers of Trachelomonas reticulata Klebs. It contained such species as Chrysococcus rufescens Klebs, Cryptochrysis commutata Pascher, Chroomonas acuta Utermohl, Euglena mutabilis Schmitz, Trachelomonas rugulosa Stein, Phacus hispidula (Eichw.) Lemm., Cryptoglena pigra Ehrenb., Astasia Klebsii, Lemm., Menoidium tortuosum Stokes, and Trigonomonas compressa Klebs. These are forms which the writer has not found to be widespread in natural bodies of water. There was also a species of Trachelomonas and one of Menoidium not referable to described species. On the other hand, there were forms which were very common to decaying submerged vegetation,

as Chilomonas paramecium Ehrenberg, and such cosmopolitan forms as Cyclidium glaucoma O.F.M.

The large species list for this situation might be accounted for by the chances for frequent entry of water from the pond. But the development therein, in large numbers, of forms not common to, or not observed in the pond at all, argues for the existence of specific microclimatic conditions in the tree hole favorable for such organisms. That this is the case is further borne out by other tree holes. Holes Numbers 10 and 12 had 16 and 34 species respectively, and were so high that only rain water could trickle in. Hole Number 10 was the breeding place for four species of mosquitoes, so it is surprising that its list of organisms comprises even 16 species. Its organisms were not unusual, but most of the Chlamydomonas and Chlorogonium in it were colorless or nearly so, and in a large population of Blepharisma undulans Stein, the pink color was either completely gone or partly so. Hole Number 12, with 34 listed species showed some unusual forms as Acinetactis mirabilis Stokes, about whose validity Pascher (2) is perhaps dubious, and Dactylochlamys pisciformis Lauterborn. Green flagellates were scarce in this hole, but it contained Phacus triqueter (Ehrenb) Dujardin --- and they were all colorless or nearly so! The existence of these colorless, but apparently thriving forms of normally green flagellates is further evidence for specific microclimatic factors.

A comparison of the organisms in all holes shows a strong tendency to recur. It has been stated elsewhere (3) that only very few protozoa or flagellates might be reasonably expected in a random sample of a natural water, as a stagnant pool, or quiescent spot along a river bank. If we take the entire 29 holes examined for this study, some of the organisms occur with a

relatively high frequency. This tendency is shown in Table 1. While the percentage of occurrence in samples is not strikingly large, it is nevertheless decided, and is further corroboration of some sameness in these habitats.

It is interesting to speculate on how these forms first entered tree holes which now have no connection with ground water. Upward growth of the hole may be postulated in a few cases. Upward migration of some forms in the film of water along the bark in wet seasons may also be taken into account. Some of the forms are those which Unger[4] and others have shown to be found on vegetation in the form of cysts. But for others, cysts are as yet unknown, and these could hardly be transported to the tree holes by winds or animals. Perhaps a combination of methods is the easiest way to account for entrance. A few high tree holes were investigated but none had any water in them.

It is worth noting that algae, exclusive of the flagellated forms, were largely absent from these situations. Pleurococcus was probably the most frequent, but despite the diffuse light present in some of the situations, Protococcales were almost wholly lacking, only a few diatoms were seen, and no blue green algae were recorded. It may be inferred that there was too much organic matter present for the development of most algae, since several species of algae were found in the Tupelo gum pond, and at least some of the holes admitted enough light for chlorophyll bearers.

Altogether the collection of organisms studied herein is interesting because it exhibits a tendency to be an environmental group; because of its unusual forms; because of the tendency for some of the green flagellates to lose their chlorophyll and assume a saprophytic existence; and because of the evidence which it may offer for microclimatic factors in these small environmental niches.

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## TABLE I

The Location of 26 Tree Holes Investigated for Microscopic Life, showing Location, Kind of Tree, Hydrogen ion Concentration when Observed, and Listing Those Species which occurred in 4 or More of the Holes.

| Hole No. | Location | Kind of Tree | pH | Number Species | Oxytricha sp. | Halteria grandinella | Metopus sp. | Vorticella sp. | Distigma proteus | Menoidium incurvum | Colpoda aspera | Oicomonas sp. | Menoidium sp. | Chilodonella uncinatus | Cinetochilum margaritaceum | Cyclidium glaucoma | Astasia inflata | Chlamydomonas sp. | Bodo globosus | Hexamitus crassa | Polytoma uvella | Trachelomonas volvocina |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Alabama | White Oak | | 11 | x | | x | x | x | | | | | | | | x | | | | | |
| 2 | " | Blackjack Oak | | 2 | | | | | | | | x | | x | | | | | | | | |
| 3 | " | Tupelo Gum | 6.0 | 61 | x | x | x | x | x | x | | x | x | x | | x | x | x | | x | | x |
| 4 | " | " " | | 3 | | | | | | | | | | | | | | | | | x | |
| 5 | " | " " | | 1 | | | | | | | | x | | | | | | | | | | |
| 6 | " | " " | 6.2 | 13 | x | x | | x | | | | | | | x | | | | | | x | |
| 7 | " | " " | 6.0 | 6 | | | | x | | | | | | | x | | x | | | | | |
| 8 | " | " " | | 2 | | | | | | x | | | | | | | | | | | | |
| 9 | " | " " | | 10 | | | x | | x | x | | | | | | | | | | x | | |
| 10 | " | Black Gum | 6.1 | 16 | x | x | | | | | x | | | | | | | | | | | |
| 11 | " | Tupelo Gum | | 4 | | | | | | | | | | | x | | | x | | | | |
| 12 | " | " " | 6.0 | 35 | x | x | x | x | x | x | x | x | x | x | | x | | | x | x | | x |
| 13 | " | " " | 5.8 | 8 | | | x | x | | | | | | | | | | | | | | x |
| 14 | " | " " | | 12 | x | | | | | x | | | | | | x | | | | x | | x |
| 15 | " | " " | 6.0 | 6 | | x | | x | | x | | | x | | | | | | | | | |
| 16 | " | Sweet Gum | | 35 | x | | | | | | | | | | | | | | | | | |
| 17 | " | Tupelo Gum | 6.1 | 8 | | x | | | x | | | | | | | | | x | x | | | |
| 18 | " | White Oak | | 2 | | | | | x | | | | x | x | x | x | x | x | | | x | |
| 19 | Ohio | Maple | 7.0 | 9 | | | | | x | | x | | | | | | | | | | | |
| 20 | " | Maple | | 4 | | | | x | | | x | | | | | | | | | | | |
| 21 | " | American Elm | 7.2 | 1 | | | | | | | x | x | | | | | | | | | | |
| 22 | " | " " | 7.2 | 6 | | | | | | | | | | | | | | | x | | x | |
| 23 | Kentucky | Red Oak | | 6 | | | | | | | | | | | | | | | | | | |
| 24 | Ohio | American Elm | 7.1 | 1 | | | | | | | | | | | | | | | | | | |
| 25 | " | Sycamore | 7.1 | 27 | | | x | x | | | | | | | | | | | | | | |
| 26 | " | Maple | 7.1 | 1 | | | | | | | | | | | | | | | | | | |